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Effects of anticholinergic drugs on rabbit efferent phrenic discharges[1]

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ABSTRACT In conscious, vagotomized, curarized, and artificially-ventilated rabbits, the efferent phrenic discharges were recorded. When scopolamine, atropine, pirenzepine or AF-DX 116 (11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*[2,3-6] [1,4]benzodiazepine-6-one) was injected into the cerebello-medullary cistern, the frequency and voltage of phrenic discharges were decreased ($P<0.05$) by scopolamine (0.5 mg · kg^{-1}) and pirenzepine (0.5 mg · kg^{-1}), but were increased ($P<0.01$) by atropine (0.05 mg · kg^{-1}) and AF-DX 116 (0.1 mg · kg^{-1}). It is probable that scopolamine inhibits the respiratory center by blocking the M_1 cholinergic receptors while atropine excites the respiratory center blocking the M_2 cholinergic receptors.

KEY WORDS respiratory center; phrenic nerve; electrophysiology; scopolamine; pirenzepine; atropine; benzodiazepines

It was established that atropine (Atr) stimulates the respiratory center, but it was inconsistent whether the effect of scopolamine (Scop) is stimulatory or inhibitory. It was reported that the frequency of phrenic discharges was increased by Scop[1]. Scop is sometimes used to treat respiratory failure in China[2]. We recently studied the efferent phrenic discharges to explore the excitability of the respiratory center when Scop, Atr, pirenzepine (PZ), and AF-DX 116 (11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*[2,3-6] [1,4]benzodiazepine-6-one) were separetely injected into the cerebellomedullary cistern.

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MATERIALS AND METHODS

The drugs used included Scopolamine hydrobromide, atropine sulfate (Chengdu First Pharmaceutical Plant), pirenzepine (Chongqing Institute of Materia Medica), AF-DX 116 (gifted by Dr. Karl Thomae GmbH Chemisch-pharmazeutiche Fabrik, Germany), and gallamine triethiodide (Shanghai Institute of Biochemistry, Chinese Academy of Sciences). They were all dissolved in distilled water to the concentration needed, except that AF-DX 116 was dissolved in HCl 0.1 mol · L^{-1}.

The rabbits (2.6 ± SD 0.24 kg, either sex) were operated under local anesthesia with 2% lidocaine. The trachea was intubated and both vagi were severed, and gallamine triethiodide was iv to relax muscles. The lung was mechanically ventilated at a frequency of 32 · min^{-1} with a tidal volume of 30 ml. The rectal temperature was maintained at 38.5-39℃. A median incision was made on the nucha and the space between the occipital bone and the atlas was prepared for puncture through the foramen magnum into the cerebello-medullary cistern to administer drugs. To ensure the needle was in the cistern, a little cerebrospinal fluid was drawn after each puncture; the amount of drugs was limited within 0.15 ml each time.

The phrenic nerve was isolated, the phrenic impulses were displayed on the Vc-10 doubleline oscilloscope for direct visual observation and selected photography. Simultaneously, the impulses were put into type 117 nerve impulse analyzer to record the frequency and voltage of the discharges in each inspiration.

RESULTS

The effects of anticholinergic drugs on efferent phrenic discharges were observed with all the rabbits fixed on the stereolocator. In the control group ($n=7$), 2 rabbits were given nothing and the other 5 were given normal saline (amount and pH similar to those of drug solutions). The indices showed no significant changes within 1 h.

When Scop was injected into the cerebello-medullary cistern, in the 0.1 mg · kg^{-1} group ($n=5$), no apparent changes in the indices were seen. In the 0.5 mg · kg^{-1} group ($n=7$), the frequency of phrenic discharges decreased by 25 ± 21% ($P<0.05$) at 30 min, and gradually restored to normal by 1 h. A marked decrease in the voltage of phrenic discharges was found to be 13 ± 10% ($P<0.05$) at 20 min and persisted for more than 1 h.

When PZ 0.5 mg · kg^{-1} was given, the frequency of phrenic discharges decreased by 52 ± 15% at 10 min ($P<0.05$) and recovered to normal in 1 h. The voltage of phrenic discharges decreased by 38 ± 17% ($P<0.05$).

In Atr 0.05 mg · kg^{-1} group, the increase of frequency of phrenic discharges was apparent at 10 min, reached its peak (by 92 ± 30%, $P<0.01$) at 30 min and lasted more than 1 h. The increase of voltage of the discharges was evident and reached its peak (by 36 ± 28%, $P<0.05$) at 15 min. When the dosage of Atr was increased to 0.1 mg · kg^{-1}, the frequency of phrenic discharges became

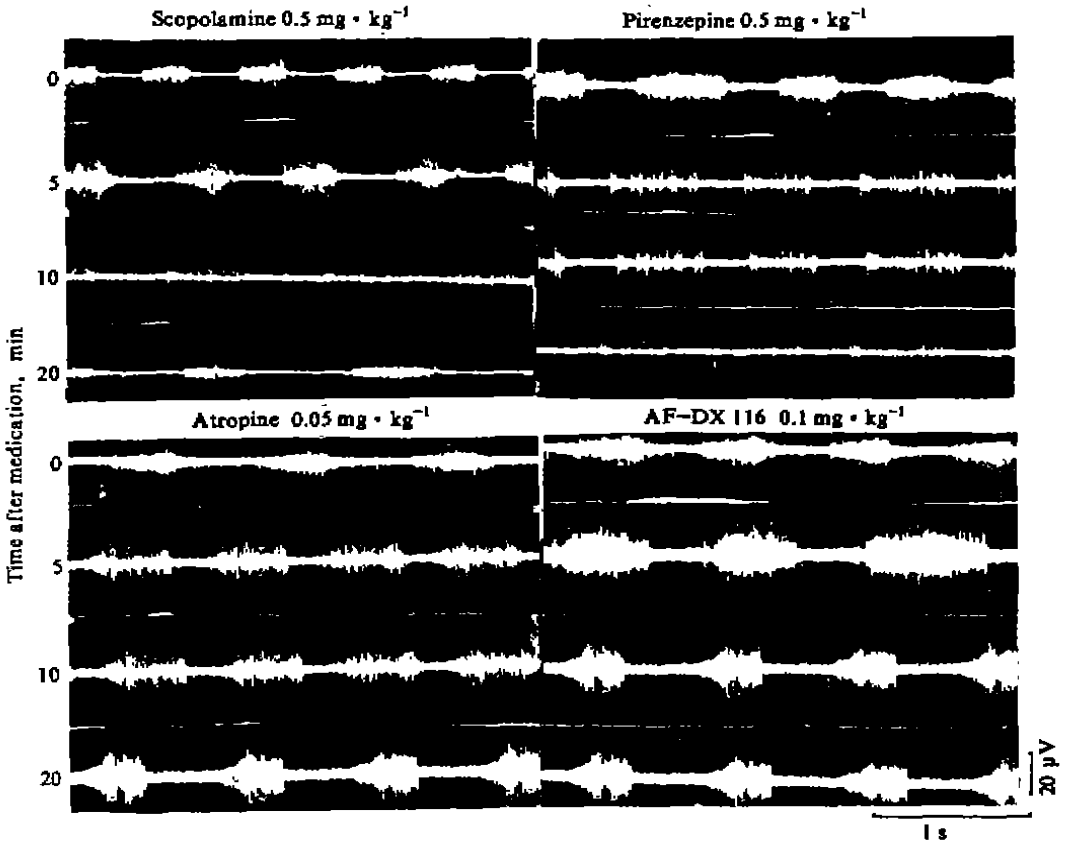

Fig 1. Oscilloscopic pictures showing the effects of anticholinergic drugs (injected into cerebello-medullary cistern) on efferent phrenic discharges in rabbits.

Tab 1. Effects of anticholinergic drugs (injected into cerebello–medullary cistern) on efferent phrenic discharges in rabbits. $n=5–7$, $\bar{x}\pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* before drugs.

Drugs (mg · kg^{-1})		0	5	10	20	30	45	60 min
		Frequency of discharge (impulses / inspiration)						
Saline		101 ± 11	106 ± 10*	101 ± 5*	102 ± 10*	97 ± 10*	96 ± 16*	92 ± 20*
Scopolamine	0.1	96 ± 15	98 ± 18*	99 ± 25*	105 ± 41*	95 ± 49*	93 ± 51*	82 ± 50*
	0.5	102 ± 7	96 ± 26*	92 ± 32*	78 ± 22**	78 ± 23**	82 ± 38*	96 ± 46*
Pirenzepine	0.5	64 ± 13	33 ± 21**	32 ± 23**	41 ± 29*	34 ± 33*	35 ± 37*	53 ± 46*
Atropine	0.05	48 ± 14	60 ± 18**	72 ± 31**	87 ± 27**	92 ± 30***	91 ± 42**	82 ± 47*
AF–DX 116	0.1	47 ± 9	75 ± 32**	104 ± 68**	116 ± 59**	140 ± 93**	145 ± 95**	126 ± 89**
		Voltage of discharge (μV)						
Saline		15.6 ± 0.6	15.8 ± 0.8*	15.4 ± 1.1*	15.4 ± 1.5*	15.2 ± 1.5*	15.1 ± 1.3*	15.0 ± 0.9*
Scopolamine	0.1	24.0 ± 6.0	24.8 ± 6.8*	24.8 ± 6.0*	23.6 ± 5.8*	22.4 ± 6.6*	20.8 ± 6.0*	19.6 ± 7.7*
	0.5	22.0 ± 6.8	19.2 ± 8.4*	19.8 ± 7.6**	19.2 ± 6.4**	19.4 ± 7.0**	19.2 ± 5.4**	20.8 ± 5.2**
Pirenzepine	0.5	20.0 ± 5.2	20.7 ± 8.6*	21.0 ± 11.4*	21.0 ± 9.6*	20.0 ± 8.8*	13.2 ± 6.6**	17.6 ± 6.7*
Atropine	0.05	15.0 ± 7.5	19.0 ± 9.4**	19.0 ± 8.8**	22.0 ± 9.2**	19.7 ± 10.8*	18.0 ± 8.0*	14.0 ± 5.0*
AF–DX 116	0.1	16.7 ± 5.5	25.6 ± 13.5*	28.7 ± 25.7*	35.3 ± 32.8*	44.0 ± 36.6*	34.0 ± 32.7*	34.0 ± 32.7*

too fast to be read by the instrument (>200 impulses / inspiration).

In AF–DX 116 0.1 mg · kg^{-1} group, the frequency of phrenic discharges increased by 138 ± 73% ($P<0.01$) for more than 1 h; the voltage of the discharges increased by 114 ± 40% ($P<0.05$).

DISCUSSION

The phrenic discharge reflects the excitation of respiratory center[3]. In our experiment the frequency and the voltage of phrenic discharges were stable in the control group, indicating that the method employed is reliable.

The frequency and voltage of phrenic discharges were decreased by Scop, establishing that it inhibited the respiratory center. As PZ, the selective blocking agent of M_1 cholinergic receptors[4–5], exerted similar effects as Scop, the inhibitory effect of these 2 drugs on respiration is probably related to their common blocking effect on M_1 cholinergic receptors in the respiratory center.

Analogically, since Atr excited the respiratory center as did AF–DX 116, the selective M_2 cholinergic receptor blocking agent[6], it is likely that they acted on the respiratory center by blocking the M_2 cholinergic receptors.

Recently, our receptor binding assays with [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) and [^{3}H]pirenzepine ([^{3}H]PZ) demonstrated the presence of M_1 and M_2 subtypes of M cholinergic receptors in the pons and medulla, and showed that Scop had stronger affinity for M_1 than for M_2 cholinergic receptors, while Atr had stronger affinity for M_2 than for M_1 cholinergic receptors[7]. These results support our explanation that the inhibitory effect of Scop respiratory center is related to the blocking of its M_1 cholinergic receptors and the excitatory effect of Atr is related to the blocking of its M_2 cholinergic receptors.

REFERENCES

1 Xu NS, Pan LH, Zhang JR. Effect of intraventricular application of neurotransmitters to rabbits on high-frequency oscillation (HFO) in phrenic discharge. *Acta Physiol Sin* 1983; 35: 193

2 Yang KT, Hsu CK, T'ung KF. Scopolamine in emergency treatment of respiratory failure in very severe encephalitis B. *Chin Med J* 1973; **53**: 283
3 Pan LH, Zhan WZ, Zhang JR. Relation between high-frequency oscillation in phrenic discharge and duration of inspiration. *Acta Physiol Sin* 1982; **34**: 49
4 Hammer R, Berrie CP, Birdsall NJM, Burgen ASV, Hulme EC. Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* 1980; **283**: 90
5 Bian CF, Xing SH, Shao LN, Qin W. Central inhibitory effects of pirenzepine. *Acta Pharmacol Sin* 1987; **8**: 396
6 Giraldo E, Hammer R, Landisky H. Distribution of muscarinic receptor subtypes in rat brain as determined in binding studies with AF-DX 116 and pirenzepine. *Life Sci* 1987; **40**: 833
7 Zheng JL, Bian CF. Analysis of muscarinic receptor subtypes in rat brain stem. *Acta Acad Med Xuzhou* 1991; **11**: 5

抗胆碱药对兔膈神经放电的影响

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提要　于清醒、肌松、双侧迷走神经切断的兔，记录膈神经放电，小脑延髓池注射药物．东莨菪碱 0.5 mg·kg^{-1} 和哌仑西平 0.5 mg·kg^{-1} 使膈神经放电频率减少，电压降低($P<0.05$)，阿托品 0.05 mg·kg^{-1} 和 AF-DX 116 0.1 mg·kg^{-1} 使放电频率增加，电压增大($P<0.01$)，结果显示东莨菪碱抑制呼吸中枢，可能与其阻断 M_1 受体有关，阿托品的呼吸中枢兴奋作用，可能与其阻断 M_2 受体有关．

关键词　呼吸中枢；膈神经；电生理；东莨菪碱；哌仑西平；阿托品；苯并二氮䓬类

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Effects of nimodipine on *l*-glutamate-induced seizures and Ca^{2+} influx in hippocampus in freely moving rats

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ABSTRACT　Seizure (EEG) was studied in rats unilaterally injected in the dorsal hippocampus with *l*-glutamate (Glu). Extracellular Ca^{2+} content [$(Ca^{2+})_e$] in the injected area was assessed by brain microdialysis coupled to automatic atomic absorption spectrophotometry. In this experimental epileptic model, an inhibition of Glu-stimulated epileptic activity and a fall in $(Ca^{2+})_e$ by nimodipine (Nim, 100 μg·kg^{-1}) were seen. The spike- and wave-burst frequency was reduced from 30 to 5 bursts·min^{-1} ($P<0.01$, $n=8$). Nim 25 and 50 μg·kg^{-1}, without anticonvulsant activity, did not prevent the drop in $(Ca^{2+})_e$. These results indicate that Nim exerts an antiepileptic effect on Glu-induced epilepsy. The mechanisms may be involved in blocking Ca^{2+} influx into neurons.

KEY WORDS　calcium; nimodipine; spectrophotometry; epilepsy

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Ca^{2+} influx into neurons seems to play an important role in excitatory amino acids-induced epileptic activity[1]. Experimental studies exploring antiepileptic activity of calcium antagonists revealed anticonvulsive properties of flunarizine[2] and verapamil[3]. Nimodipine (Nim), so far studied for its effects, was only restricted to the cerebral